

WHAT IS CLAIMED IS:

- 1 1. A method for detecting the presence or activity of a translocation promoting agent,
 2 wherein said translocation promoting agent is measured by:
 - 3 (a) contacting a biological sample from a mammal in which the presence or
 4 activity of said translocation promoting agent is suspected with a binding partner of said
 5 translocation promoting agent under conditions that allow binding of said translocation
 6 promoting agent to said binding partner to occur; and
 - 7 (b) detecting whether binding has occurred between said translocation
 8 promoting agent from said sample and the binding partner; wherein the detection of
 9 binding indicates the presence or activity of said translocation promoting agent in said
 10 sample; wherein said translocation promoting agent being capable of promoting the
 11 translocation of macrophage-tropic virus through the membrane of a target CD4⁺ cell, said
 12 translocation promoting agent comprising a material selected from the group consisting of a
 13 protein, active fragments thereof, agonists thereof, mimics thereof, and combinations
 14 thereof, said translocation promoting agent having the following characteristics:
 - 15 (i) the agent is present in or on or proximal to the cell membrane of
 16 said target cell;
 - 17 (ii) the agent acts in conjunction with CD4 in connection to said
 18 translocation; and
 - 19 (iii) the agent is in association with a G-protein, wherein said
 20 association can facilitate an intracellular signal.
- 1 2. A method for detecting the presence and activity of a polypeptide ligand associated
 2 with a given invasive stimulus in mammals comprising detecting the presence or activity of
 3 a translocation promoting agent according to the method of Claim 1, wherein detection of
 4 the presence or activity of the translocation promoting agent indicates the presence and
 5 activity of a polypeptide ligand associated with a given invasive stimulus in mammals.
- 1 3. The method of Claim 1 wherein said intracellular signal results in an increase in
 2 levels of intracellular calcium.
- 1 4. The method of Claim 1 wherein said translocation promoting agent is a member of
 2 the transmembrane G-protein coupled receptor family.

1 5. The method of Claim 1 wherein said translocation promoting agent is derived from
2 a human cell.

1 6. The method of Claim 5 wherein the translocation promoting agent is CC-CKR5.

1 7. A method for identifying a viral envelope glycoprotein that binds a translocation
2 promoting agent, comprising:

3 (a) contacting a labeled translocation promoting agent with a viral envelope
4 glycoprotein attached to a solid support;

5 (b) washing the solid support; and

6 (c) detecting the labeled translocation promoting agent associated with the solid
7 support; wherein a viral envelope glycoprotein that binds a translocation promoting agent is
8 identified when the labeled translocation promoting agent is detected associated with the
9 solid support; wherein said translocation promoting agent is capable of promoting the
10 translocation of macrophage-tropic virus through the membrane of a target CD4⁺ cell, said
11 translocation promoting agent comprising a material selected from the group consisting of a
12 protein, active fragments thereof, agonists thereof, mimics thereof, and combinations
13 thereof, said translocation promoting agent having the following characteristics:

14 (i) the agent is present in or on or proximal to the cell membrane of
15 said target cell;

16 (ii) the agent acts in conjunction with CD4 in connection to said
17 translocation; and

18 (iii) the agent is in association with a G-protein, wherein said
19 association can facilitate an intracellular signal.

1 8. The method of Claim 7, wherein the viral envelope glycoprotein is an HIV
2 envelope glycoprotein.

1 9. The method of Claim 7, wherein the translocation promoting agent is CC-CKR5.

1 10. An assay system for screening a drug for its ability to modulate the production of a
2 translocation promoting agent, comprising:

3 (a) culturing a mammalian cell that has been inoculated with a drug;

4 (b) harvesting a supernatant from said cell; and

- 1 (c) examining said supernatant for the presence of said translocation promoting
2 agent wherein an increase or a decrease in a level of said translocation promoting agent
3 indicates the ability of the drug to modulate the activity of said translocation promoting
4 agent, said translocation promoting agent capable of promoting the translocation of
5 macrophage-tropic virus through the membrane of a target CD4⁺ cell, said translocation
6 promoting agent comprising a material selected from the group consisting of a protein,
7 active fragments thereof, agonists thereof, mimics thereof, and combinations thereof, said
8 translocation promoting agent having the following characteristics:
- 9 (i) the agent is present in or on or proximal to the cell membrane of
10 said target cell;
- 11 (ii) the agent acts in conjunction with CD4 in connection to said
12 translocation; and
- 13 (iii) the agent is in association with a G-protein, wherein said
14 association can facilitate an intracellular signal.

- 1 11. A test kit for the demonstrating the presence of a translocation promoting agent in a
2 eukaryotic cell, comprising:
- 3 (a) a predetermined amount of a detectably labelled specific binding partner of
4 a translocation promoting agent;
- 5 (b) other reagents; and
- 6 (c) directions for use of said kit; wherein said translocation promoting agent is
7 capable of promoting the translocation of macrophage-tropic virus through the membrane of
8 a target CD4⁺ cell, said translocation promoting agent comprising a material selected from
9 the group consisting of a protein, active fragments thereof, agonists thereof, mimics
10 thereof, and combinations thereof, said translocation promoting agent having the following
11 characteristics:
- 12 (i) the agent is present in or on or proximal to the cell membrane of
13 said target cell;
- 14 (ii) the agent acts in conjunction with CD4 in connection to said
15 translocation; and
- 16 (iii) the agent is in association with a G-protein, wherein said
17 association can facilitate an intracellular signal.

1 12. The test kit of Claim 11 further comprising a predetermined amount of a
2 translocation promoting agent.

1 13. The test kit of Claim 11 wherein said detectably labelled specific binding partner of
2 a translocation promoting agent is selected from the group consisting of polyclonal
3 antibodies to the translocation promoting agent, monoclonal antibodies to the translocation
4 promoting agent, fragments thereof, and mixtures thereof.

1 14. A method of preventing and/or treating cellular debilitations, derangements and/or
2 dysfunctions and/or other disease states in mammals, comprising administering to a
3 mammal a therapeutically effective amount of a material selected from the group consisting
4 of an agent capable of inhibiting the production of a translocation promoting agent, soluble
5 translocation promoting agent, antagonists to said translocation promoting agent, cognates
6 thereof, fragments thereof, and mixtures thereof, or a specific binding partner thereto, said
7 translocation promoting agent capable of promoting the translocation of macrophage-tropic
8 virus through the membrane of a target CD4⁺ cell, said translocation promoting agent
9 comprising a material selected from the group consisting of a protein, active fragments
10 thereof, agonists thereof, mimics thereof, and combinations thereof, said translocation
11 promoting agent having the following characteristics:
12 (i) the agent is present in or on or proximal to the cell membrane of
13 said target cell;
14 (ii) the agent acts in conjunction with CD4 in connection to said
15 translocation; and
16 (iii) the agent is in association with a G-protein, wherein said
17 association can facilitate an intracellular signal.

1 15. The method of Claim 14 wherein said disease states include AIDS, and related
2 conditions.

1 16. The method of Claim 14 wherein said intracellular signal results in an increase in
2 levels of intracellular calcium.

1 17. The method of Claim 14 wherein said translocation promoting agent is a member of
2 the transmembrane G-protein coupled receptor family.

1 18. The method of Claim 14 wherein said translocation promoting agent is CC-CKR5.

1 19. A pharmaceutical composition for the treatment of cellular debilitation,

2 derangement and/or dysfunction in mammals, comprising:

3 (a) a therapeutically effective amount of a material selected from the group
4 consisting of an agent capable of inhibiting the production of a translocation promoting
5 agent, soluble translocation promoting agent, antagonists to said translocation promoting
6 agent, cognates thereof, fragments thereof, and mixtures thereof, or a specific binding
7 partner thereto; and

8 (b) a pharmaceutically acceptable carrier; wherein said translocation promoting
9 agent is capable of promoting the translocation of macrophage-tropic virus through the
10 membrane of a target CD4⁺ cell, said translocation promoting agent comprising a material
11 selected from the group consisting of a protein, active fragments thereof, agonists thereof,
12 mimics thereof, and combinations thereof, said translocation promoting agent having the
13 following characteristics:

14 (i) the agent is present in or on or proximal to the cell membrane of
15 said target cell;

16 (ii) the agent acts in conjunction with CD4 in connection to said
17 translocation; and

18 (iii) the agent is in association with a G-protein, wherein said
19 association can facilitate an intracellular signal.

1 20. The composition of Claim 19 wherein said translocation promoting agent is a
2 member of the transmembrane G-protein coupled receptor family.

1 21. The composition of Claim 20 wherein said translocation promoting agent is CC-
2 CKR5.

1 22. A transgenic non-human mammal comprising a DNA construct containing a human
2 CD4 gene and a DNA construct containing human CC-CKR-5 gene wherein both CD4
3 protein and CC-CKR-5 protein are expressed by said non-human mammal.

- 1 23. The transgenic non-human mammal of Claim 22, wherein the DNA construct for
2 the human CD4 gene contains a T cell-specific transcriptional enhancer element.
- 1 24. The transgenic non-human mammal of Claim 22, wherein said non-human mammal
2 is a mouse.
- 1 25. The transgenic non-human mammal of Claim 24, wherein said mouse lacks
2 endogenous CD4.
- 1 26. The transgenic non-human mammal of Claim 25 wherein said lack of endogenous
2 CD4 is due to selective inactivation of the CD4 gene by gene targeting.
- 1 27. A cell that is transfected with CD4 and a translocating promoter, wherein both CD4
2 and the translocation promoting agent are expressed by said cell, and wherein said cell is
3 measurably susceptible to infection by a virus pseudotyped with a macrophage-tropic
4 envelope.
- 1 28. The cell of Claim 27 wherein said translocating promoter is CC-CKR-5.
- 1 29. The cell of Claim 28 wherein said cell is attached to a solid support matrix.
- 1 30. The cell of Claim 29 wherein said cell is a mammalian cell.
- 1 31. The cell of Claim 30 wherein said mammalian cell is a human cell.
- 1 32. The cell of Claim 27 wherein said human cell is a embryonic kidney 293T cell.
- 1 33. A cell that is transfected with CD4 and a mimic of the translocation promoter agent
2 wherein both CD4 and the translocation promoting agent are expressed by said cell, and
3 said mimic has the ability to function with CD4 and permit entry into a cell of a virus
4 pseudotyped with a macrophage-tropic envelope; wherein said cell is measurably
5 susceptible to infection by a virus pseudotyped with a macrophage-tropic envelope; and
6 wherein said mimic is a truncated chemokine receptor or a small organic molecule.

- 1 34. An antisense nucleic acid against an mRNA coding for CC-CKR5 comprising a
2 nucleic acid sequence that hybridizes to said mRNA.
- 1 35. A recombinant DNA molecule having a DNA sequence which, on transcription,
2 produces the antisense nucleic acid of Claim 34.
- 1 36. A cell line transfected with the recombinant DNA molecule of Claim 35.
- 1 37. An assay for selecting for a suspected therapeutic agent for possible use in the
2 treatment of AIDS with the use of the cell of Claim 27 which comprises
3 (a) administering a potential therapeutic agent to said cell;
4 (b) infecting said cell with a virus pseudotyped with a macrophage-tropic
5 envelope;
6 (c) measuring the ability of said cell to resist said infection; and
7 (d) selecting the potential therapeutic agent when the measured ability of said
8 cell to resist said infection is statistically greater in the presence of said potential therapeutic
9 agent than in the absence of said potential therapeutic agent; wherein said selected potential
10 therapeutic agent is a suspected therapeutic agent.
- 1 38. An assay for selecting a plausible therapeutic agent for possible use in the treatment
2 of AIDS with the use of the transgenic non-human mammal of Claim 22, comprising:
3 (a) administering a suspected therapeutic agent to the transgenic non-human
4 mammal;
5 (b) infecting said transgenic non-human mammal with a virus pseudotyped with
6 a macrophage-tropic envelope;
7 (c) measuring the ability of said transgenic non-human mammal to resist said
8 infection; and
9 (d) selecting the suspected therapeutic agent when the measured ability of said
10 transgenic mammal to resist said infection is statistically greater in the presence of said
11 suspected therapeutic agent than in the absence of said suspected therapeutic agent; wherein
12 said selected suspected therapeutic agent is a plausible therapeutic agent.

1 39. A method of filtering a biological fluid to remove a virus pseudotyped with a
 2 macrophage-tropic envelope wherein the biological fluid is passed through the cell of Claim
 3 29.

1 40. The method of Claim 39 when said biological fluid is selected from the group
 2 consisting of blood, semen, and cerebrospinal fluid.

1 41. A transformed mammalian cell that:
 2 (a) contains a gene encoding CD4;
 3 (b) contains a construct encoding a reporter gene under the regulation of an
 4 HIV LTR; and
 5 (c) that has been transduced with a retroviral vector encoding a human
 6 chemokine receptor.

1 42. The cell of Claim 41 which expresses low levels or no CXCR4 and CC-CKR5 in
 2 the absence of transduction with a retroviral vector encoding a human chemokine receptor.

1 43. The cell of Claim 41 that is a human cell.

1 44. The human cell of Claim 43 which is HOS.CD4.

1 45. The cell of Claim 41, wherein the reporter gene encodes green fluorescent protein.

1 46. The cell of Claim 41, wherein the HIV LTR is HIV-2 LTR.

1 47. The cell of Claim 41, wherein the human chemokine receptor is selected from the
 2 group consisting of CC-CKR1, CC-CKR2A, CC-CKR2B, CC-CKR-3, CC-CKR-4,
 3 CC-CKR5, and CXC-CR4.

1 48. A method for identifying a human chemokine receptor that facilitates the infection
 2 of a particular HIV strain into the transformed mammalian cell of Claim 41 comprising:
 3 (a) infecting the cell with a primary HIV strain; and

4 (b) detecting the reporter gene; wherein the human chemokine receptor is
5 identified when the reporter gene is detected above the background value determined in the
6 absence of performing step (a).

1 49. The method of Claim 48, wherein the reporter gene encodes green fluorescent
2 protein.

1 50. The method of Claim 49, wherein said detecting is performed by FACS analysis.

1 51. The method of Claim 48, wherein the human chemokine receptor is selected from
2 the group consisting of CC-CKR1, CC-CKR2A, CC-CKR2B, CC-CKR-3, CC-CKR-4, CC-
3 CKR5, and CXC-CR4.

1 52. The method of Claim 48, wherein the particular HIV strain is a primary HIV-1
2 strain.

1 53. A method of identifying a drug that interferes with the translocation of HIV into the
2 transformed mammalian cell of Claim 41 comprising:

- 3 (a) administering a potential drug to the cell;
4 (b) infecting the cell with a primary HIV strain; and
5 (c) detecting the reporter gene; wherein the reporter gene is detected in the
6 absence of the drug, indicating that the HIV strain is translocated into the cell; and
7 wherein the potential drug is identified as a drug when the reporter gene is either not
8 detected, or is detected in a lesser amount in the presence of the drug.

1 54. The method of Claim 53, wherein the reporter gene encodes green fluorescent
2 protein.

1 55. The method of Claim 53, wherein said detecting is performed by FACS analysis.

1 56. The method of Claim 53, wherein the human chemokine receptor is selected from
2 the group consisting of CC-CKR1, CC-CKR2A, CC-CKR2B, CC-CKR-3, CC-CKR-4,
3 CC-CKR5, and CXC-CR4.

- 1 57. A method of identifying an antibody that interferes with the translocation of HIV
2 into the transformed mammalian cell of Claim 41 comprising:
3 (a) administering an antibody to the cell;
4 (b) infecting the cell with a primary HIV strain; and
5 (c) detecting the reporter gene; wherein the reporter gene is detected in the
6 absence of the antibody, indicating that the HIV strain is translocated into the cell; and
7 wherein the potential antibody is identified as an antibody that interferes with the
8 translocation of HIV when the reporter gene is either not detected, or is detected in a lesser
9 amount in the presence of the antibody; and wherein the antibody is selected from the group
10 consisting of an antibody to HIV, an antibody to CD4 and an antibody to the translocation
11 promoting agent
- 1 58. The method of Claim 57, wherein the reporter gene encodes green fluorescent
2 protein.
- 1 59. The method of Claim 58, wherein said detecting is performed by FACS analysis.
- 1 60. The method of Claim 57, wherein the human chemokine receptor is selected from
2 the group consisting of CC-CKR1, CC-CKR2A, CC-CKR2B, CC-CKR-3, CC-CKR-4, CC-
3 CKR5, and CXC-CR4.
- 1 61. A nucleic acid encoding a chimeric translocation promoting agent, wherein the
2 chimeric translocation promoting agent is a chemokine receptor having an epitope tag in its
3 amino-terminal extracellular domain.
- 1 62. A nucleic acid of claim 61, wherein the chemokine receptor is CC-CKR5.
- 1 63. A nucleic acid of claim 62, wherein the chimeric translocation promoting agent
2 comprises the amino acid sequence of SEQ ID NO:6 in its amino-terminal extracellular
3 domain.
- 1 64. An expression vector comprising the nucleic acid of Claim 61.

1 65. The expression vector of Claim 64, wherein the nucleic acid encodes a chimeric
2 translocation promoting agent comprising the chemokine receptor CC-CKR5 having an
3 amino acid sequence of SEQ ID NO:6 in its amino-terminal extracellular domain.

1 66. A method of making an identifiable cell that has the chimeric translocation
2 promoting agent in its cell membrane comprising:
3 (a) transfecting a cell with the expression vector of Claim 64; and
4 (b) detecting the epitope tag with an antibody that recognizes the epitope tag;
5 wherein said detecting identifies the cell as having the chimeric translocation promoting
6 agent in its cell membrane.

1 67. The method of Claim 66, wherein the chemokine receptor is CC-CKR5 comprising
2 an amino acid sequence of SEQ ID NO:6 in its amino-terminal extracellular domain; and
3 wherein the antibody is anti-influenza (HA) monoclonal antibody.

1 68. A chimeric translocation promoting agent comprising a chemokine receptor having
2 an epitope tag in its amino-terminal extracellular domain.

1 69. The chimeric translocation promoting agent of Claim 68, wherein the chemokine
2 receptor is CC-CKR5 comprising an amino acid sequence of SEQ ID NO:6 in its amino-
3 terminal extracellular domain.